

§Appl. No. 10/088,356  
Amdt. dated February 17, 2004  
Reply to Office Action of, October 14, 2003

**Listing of Claims:**

Please **amend** the claims as follows:

**Claim 1** (Cancelled)

**Claim 2** (Withdrawn) The method of claim 1, further comprising  
f) confirming that the mutation renders *M. marinum* less virulent.

**Claim 3** (Withdrawn) A method of constructing an avirulent *M. marinum* bacterium,  
comprising mutagenizing an *M. marinum* virulence gene identified by the method of claim 1.

**Claim 4** (Withdrawn) A avirulent *M. marinum* bacterium, produced by the method of  
claim 3.

**Claim 5** (Withdrawn) An avirulent *M. marinum* bacterium, in which one or more genes  
comprising a nucleic acid of SEQ ID Nos: 4, 13, 23, 25, or 31 is mutated.

**Claim 6** (Currently Amended) A method for identifying a virulence gene of *M.*  
*tuberculosis*, comprising ~~identifying a virulence gene of *M. marinum* bacterium according to the~~  
~~method of claim 1, and further comprising,~~

a) mutagenizing an *M. marinum* bacterium by introducing into the bacterium a  
plasmid which comprises a signature-tagged transposon, whereby the transposon integrates into  
and disrupts a gene in the bacterium,

b) introducing the mutagenized bacterium into a host susceptible to infection  
thereof.

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c) identifying a bacterium which comprises a signature tagged transposon and which exhibits reduced viability in the host, compared to a non-mutagenized *M. marinum* bacterium,

d) cloning and/or sequencing a nucleic acid sequence which flanks the integrated transposon in said identified bacterium, and

e) identifying a wild type *M. marinum* gene which comprises at least a portion of said flanking sequence.

f) comparing said flanking nucleic acid sequence to a databank of *M. tuberculosis* nucleic acid sequences, and/or comparing the sequences of peptides which are coded for by said flanking sequences to a known *M. tuberculosis* protein database, and

g) identifying an *M. tuberculosis* gene which comprises a sequence that is substantially identical to said flanking sequences.

**Claim 7 (Previously Presented)** A method for generating an avirulent *M. tuberculosis* bacterium, comprising mutagenizing an *M. tuberculosis* virulence gene identified by the method of claim 6.

**Claim 8 (Previously Presented)** An avirulent *M. tuberculosis* bacterium, produced by the method of claim 7.

**Claim 9 (Previously Presented)** An avirulent *M. tuberculosis* bacterium, in which one or more of genes Rv0822c, Rv3137, or Rv2348c, is mutated to render the *M. tuberculosis* bacterium less virulent.

**Claim 10 (Previously Presented)** An avirulent *M. tuberculosis* bacterium of claim 9, in which gene Rv0822c is mutated.

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**Claim 11** (Cancelled)

**Claim 12** (Cancelled)

**Claim 13** (Cancelled)

**Claim 14** (Cancelled)

**Claim 15** (Cancelled)

**Claim 16** (Cancelled)

**Claim 17** (Cancelled)

**Claim 18** (Cancelled)

**Claim 19** (Cancelled)

**Claim 20** (Cancelled)

**Claim 21** (Previously Presented) An avirulent *M. tuberculosis* bacterium of claim 9, in which gene Rv3137 is mutated.

**Claim 22** (Previously Presented) An avirulent *M. tuberculosis* bacterium of claim 9, in which gene Rv2348c is mutated.

**Claim 23** (Cancelled)

**Claim 24** (Cancelled)

**Claim 25** (Previously Presented) An avirulent *M. tuberculosis* bacterium of claim 9, in

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which gene Rv2181, Rv1954c, Rv0987, Rv3268, or Rv2610c is mutated.

**Claim 26** (Cancelled)  
**Claim 27** (Cancelled)  
**Claim 28** (Cancelled)  
**Claim 29** (Cancelled)  
**Claim 30** (Cancelled)  
**Claim 31** (Cancelled)  
**Claim 32** (Cancelled)  
**Claim 33** (Cancelled)  
**Claim 34** (Cancelled)  
**Claim 35** (Cancelled)  
**Claim 36** (Cancelled)  
**Claim 37** (Cancelled)  
**Claim 38** (Cancelled)  
**Claim 39** (Cancelled)  
**Claim 40** (Cancelled)

**Claim 41** (Withdrawn) A pharmaceutical composition, comprising an avirulent *M. marinum* bacterium of claim 5 and a pharmaceutically acceptable carrier.

**Claim 42** (Withdrawn) An attenuated *M. marinum* vaccine, comprising an avirulent *M. marinum* bacterium of claim 5 and a pharmaceutically acceptable carrier.

**Claim 43** (Previously Presented) A pharmaceutical composition, comprising an avirulent *M. tuberculosis* bacterium of claim 9 and a pharmaceutically acceptable carrier.

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**Claim 44 (Previously Presented)** An attenuated *M. tuberculosis* vaccine, comprising an avirulent *M. tuberculosis* bacterium of claim 9 and a pharmaceutically acceptable carrier.

**Claim 45 (Previously Presented)** An attenuated *M. tuberculosis* vaccine, comprising an avirulent *M. tuberculosis* bacterium which comprises one or more mutations in one or more virulence genes identified by the method of claim 7 and a pharmaceutically acceptable carrier.

**Claim 46 (Withdrawn)** A method to elicit an immune response in a fish in need of such treatment, comprising administering to said fish an avirulent *M. marinum* bacterium of claim 5.

**Claim 47 (Currently Amended)** A method to elicit an immune response in a patient ~~in need of such treatment~~, comprising administering to said patient an avirulent *M. tuberculosis* bacterium of claim 9.

**Claim 48 (Cancelled)**

**Claim 49 (Cancelled)**

**Claim 50 (Withdrawn)** A method for isolating a mutagenized *M. marinum* bacterium which exhibits reduced virulence in a host susceptible to infection thereof compared to a non-mutagenized *M. marinum* bacterium, comprising integrating a tagged transposon into the DNA of a *M. marinum* bacterium in a manner effective to produced reduced virulence, and isolating said mutagenized bacterium.

**Claim 51 (Cancelled)**

**Claim 52 (Cancelled)**

**Claim 53 (Cancelled)**

**Claim 54 (Cancelled)**

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**Claim 55** (Cancelled)  
**Claim 56** (Cancelled)  
**Claim 57** (Cancelled)  
**Claim 58** (Cancelled)  
**Claim 59** (Cancelled)  
**Claim 60** (Cancelled)  
**Claim 61** (Cancelled)  
**Claim 62** (Cancelled)  
**Claim 63** (Cancelled)  
**Claim 64** (Cancelled)  
**Claim 65** (Cancelled)  
**Claim 66** (Cancelled)  
**Claim 67** (Cancelled)  
**Claim 68** (Cancelled)  
**Claim 69** (Cancelled)  
**Claim 70** (Cancelled)  
**Claim 71** (Cancelled)  
**Claim 72** (Cancelled)  
**Claim 73** (Cancelled)  
**Claim 74** (Cancelled)  
**Claim 75** (Cancelled)  
**Claim 76** (Cancelled)

**Claim 77** (Withdrawn) An avirulent *M. marinum* bacterium, in which a gene has been disrupted adjacent to a nucleic acid of SEQ ID NOs: 4, 13, 23, 25, or 31.

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**Claim 78 (Withdrawn)** An isolated *M. marinum* nucleic acid comprising the oligonucleotide of SEQ ID NOs: 4, 13, 23, 25, or 31, or a fragment or variant thereof; or which is complementary to, or which can hybridize under high stringency conditions to, at least a portion of said isolated nucleic acid or variant thereof.

**Claim 79 (Withdrawn)** A method to identify an agent which reduces the ability of an *M. tuberculosis* bacterium to survive in a host, comprising

- a) overexpressing one of the following *M. tuberculosis* genes: Rv0822c, Rv3137 or Rv2348c in an *M. tuberculosis* bacterium,
- b) exposing said bacterium to a putative agent, and
- c) determining if the agent reduces the viability or growth of a wild type bacterium, but not the bacterium which overexpresses said gene, in a host.

**Claim 80 (Withdrawn)** An antibody against a polypeptide encoded by one of the following *M. tuberculosis* genes: Rv0822c, Rv3137 or Rv2348c.

**Claim 81 (Withdrawn)** An antibody against a peptide encoded by one of the following *M. marinum* polynucleotides: SEQ ID NOs: 4, 13, 23, 25, or 31.

**Claim 82 (Withdrawn)** An avirulent *M. marinum* bacterium, in which a gene comprising a nucleic acid that is at least 95% identical to SEQ ID NO: 4 is mutated.

**Claim 83 (Withdrawn)** An avirulent *M. marinum* bacterium of claim 82, wherein the nucleic acid comprises nucleotides 19 to 129 of SEQ ID NO: 4.

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**Claim 84 (Previously Presented)** An avirulent *M. tuberculosis* bacterium, in which a polyketide gene is mutated to render the *M. tuberculosis* bacterium less virulent.

**Claim 85 (Previously Presented)** An avirulent *M. tuberculosis* bacterium of claim 84, in which the mutated polyketide gene is pks6 (Rv0405).

**Claim 86 (Previously Presented)** An avirulent *M. tuberculosis* bacterium of claim 84, in which the mutated polyketide gene is pks9 (Rv1664).

**Claim 87 (Previously Presented)** An avirulent *M. tuberculosis* bacterium of claim 84, in which the mutated polyketide gene is pks8 (Rv1662).

**Claim 88 (Previously Presented)** An avirulent *M. tuberculosis* bacterium of claim 84, in which the mutated polyketide gene is pks1 (Rv2946c).

**Claim 89 (Previously Presented)** An avirulent *M. tuberculosis* bacterium of claim 84, wherein said polyketide gene sequence is at least 95% identical to SEQ ID NO: 8.